Biochimica et Biophysica Acta xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

Multiple paths to peroxisomes: Mechanism of peroxisome maintenance in mammals☆

Rong Hua, Peter K. Kim *

[a] Program in Cell Biology, Hospital for Sick Children, Toronto, ON, Canada M5G 0A4
[b] Department of Biochemistry, University of Toronto, Toronto, ON, Canada M5G 1A8

ARTICLE INFO

Article history:
Received 26 July 2015
Received in revised form 18 September 2015
Accepted 21 September 2015
Available online xxxx

Keywords:
Mammalian peroxisomes
de novo biogenesis
Growth and division
Endoplasmic reticulum
Mitochondria
Peroxisomal membrane protein

ABSTRACT

Peroxisomes are dynamic organelles that can adjust their size and number in response to cellular demand and environmental stimuli. They can propagate from pre-existing peroxisomes through growth and division, as well as *de novo* from the endoplasmic reticulum (ER). However, to what extend that these two distinct peroxisome biogenesis pathways are involved in maintaining peroxisome numbers in cycling cells is unclear. Recent studies in yeast suggest that the ER plays a direct role in the maintenance of peroxisomes. However, the role of the ER in mammalian system is under debate. In this review, we outline the recent progress in understanding the biogenesis of mammalian peroxisomes. We herein discuss some of the discrepancies in the literature and the outstanding questions in the field. This article is part of a Special Issue entitled: Peroxisomes.

1. Introduction

Peroxisomes are single membrane-bound organelles found in virtually all eukaryotic organisms. Their matrix encloses at least 50 different enzymes associated with various metabolic functions depending on species, cell types, and environmental stimuli. In mammalian cells, peroxisomes are mainly responsible for β-oxidation of very long chain fatty acids (VLCFA), detoxification of hydrogen peroxide, as well as the biosynthesis of bile acids and ether lipids, such as plasmalogens [1–3]. The importance of these catabolic and anabolic reactions is best illustrated by a group of genetically heterogeneous metabolic diseases known collectively as the peroxisomal disorders, resulting from genetic defects in peroxisomal enzymes, or peroxins, which are the genes required for their formation [4].

Peroxisomes can rapidly increase in size and number to adapt to various cellular stimuli. In mammalian cells, peroxisome proliferation is induced by the activation of a group of nuclear receptors called peroxisome-proliferator-activated receptors (PPAR) that act as lipid sensors and modulate the expression of genes associated with lipid metabolism, including genes involved in peroxisome proliferation [5–8]. Genetic and proteomic screenings in yeast [9] and mutated Chinese hamster ovary (CHO) cells [10] with impairments in peroxisomal formation have led to the identification of 32 yeast genes and 13 mammalian genes (called PEX) that are required for the formation of peroxisomes [11–13]. Mutation in any of these PEX genes leads to the development of a group of inheritable diseases known as the peroxisomal biogenesis disorders (PBDs). Defects in most of these peroxins result in membrane structures commonly called 'peroxisomal membrane ghosts' that are devoid of matrix proteins [14], reflecting that most of these peroxins are involved in the import of peroxisomal matrix protein into peroxisomes. However, deletions or non-functional mutations in PEX3, PEX16, or PEX19 in mammalian peroxins [15–22], and Pex3p or Pex19p in yeast peroxins result in the complete disappearance of peroxisomal membrane structures [23,24], implying that these peroxins are required in the early stages of peroxisome biogenesis. Other genes that were not initially identified as peroxins and later shown to act on peroxisome maintenance are dynamin-like protein 1 (DLP1/Drp1) [25–28], fission 1 (FIS1) [29,30], and mitochondrial fission factor (MFF) [31–34]. The protein products of these genes cooperate with the PEX11 family to mediate the fission of peroxisomes [13].

Despite the identification of essential components involved in the peroxisome biogenesis, the origin of peroxisomes has been a topic of

Abbreviations: CHO, Chinese hamster ovary; DLP1, dynamin-like protein 1; ER, endoplasmic reticulum; FIS1, fission 1; *H. polymorpha*, *Hansenula polymorpha*; MFF, mitochondrial fission factor; MAPL, mitochondria-anchored protein ligase; MDV, mitochondria-derived vesicle; mPTS, integral membrane targeting sequence; PAGFP, photo-activable GFP; PBD, peroxisomal biogenesis disorders; PMP, peroxisomal membrane protein; PPAR, peroxisome-proliferator-activated receptors; PTS, peroxisomal targeting signals; *S. cerevisiae*, *Saccharomyces cerevisiae*; TM, transmembrane domain; VLCFA, very long chain fatty acids; *Y. lipolytica*, *Yarrowia lipolytica*.

☆ This article is part of a Special Issue entitled: Peroxisomes.

* Corresponding author at: Program in Cell Biology, Hospital for Sick Children, 686 Bay St. RM 19.9708 Toronto, ON, Canada M5G 0A4.
E-mail address: pkim@sickkids.ca (P.K. Kim).

http://dx.doi.org/10.1016/j.bbamcr.2015.09.026
0167-4889/

debate since their first discovery in the early 1950s. Below, we review the recent data and current models for the biogenesis of mammalian peroxisomes. We will also discuss some of the discrepancies in the literature and the outstanding questions in the field. For detailed discussion on peroxisome biogenesis in other species, such as yeast and plants, we refer to other reviews in this special issue.

2. Origin of mammalian peroxisomes

2.1. Historical model

Peroxisomes were first observed in mouse renal cells by J. Rhodin using electron microscopy in 1954 [35] and by W. Bernhard & C. Rouiller in rat liver in 1956 [36]. At that time, peroxisomes were referred as the "microbodies" which is a morphological term used for describing a single-membrane bound subcellular structure with a diameter between 0.2 to 1.0 μm. The term "peroxisome" was first introduced in 1966 by C. de Duve & P. Baudhuin who isolated peroxisomes from rat liver and demonstrated that the peroxisomal matrix contains an oxidase that produces H_2O_2, as well as catalase, which is a H_2O_2-decomposing enzyme [37]. In this early characterization of peroxisomes, several electron micrographs published during the early 1960s were interpreted as showing peroxisomes in close juxtaposition with the ER [38,39]. In addition, catalase has been reported to be present in the microsomal fraction of the rat liver by T. Higashi & T. Jr. Peters in 1963 [40]. Taken together, C. de Duve & P. Baudhuin proposed in their early review that peroxisomes formed by budding from the ER [37]. This hypothesis was further supported by several subsequent electron micrographs showing that peroxisomes were surrounded by and continuous with the smooth ER in both pig intestinal cells and mouse hepatocytes [41,42].

2.2. Discovery of growth and division model

In the mid 1980s, Paul B. Lazarow and Yukio Fujiki challenged the concept of ER budding with the introduction of the "growth and division" model [43]. According to this model, peroxisomes are autonomous organelles, like mitochondria and chloroplasts, which proliferate by the fission of pre-existing peroxisomes. This postulation was based on a seminal finding that peroxisomal matrix and membrane proteins were synthesized on free polyribosomes in the cytosol and then imported post-translationally into peroxisomes [43–47]. Today, we know that most peroxisomal matrix proteins contain one of the two peroxisomal targeting signals, i.e., PTS1 or PTS2, which is recognized by the soluble receptor PEX5 or PEX7 respectively [48,49]. The cargo-loaded receptors will target directly to peroxisomes via their interaction with the docking complex at the peroxisomal membranes. After being translocated across the membranes, the cargo is then released into the matrix of peroxisome and the receptor is recycled back to the cytosol [11,49–51]. Peroxisomal membrane proteins (PMPs), on the other hand, contain the integral membrane targeting sequences (mPTS), which typically consist of a transmembrane domain flanked by charged residues on either side [50,52–54]. These mPTS are recognized by the cytosolic chaperone PEX19 that guides the PMPs to peroxisomal membranes via its interaction with the docking factor PEX3 [55–57]. For detailed discussion on peroxisomal protein targeting, we refer to reviews [11,49–51].

The growth and division of peroxisomes is mediated by the components of the elongation and fission machinery, including the PEX11 family, DLP1/Drp1, MFF and FIS1 [25–28]. During peroxisome proliferation, the PEX11 family proteins mediate the protrusion of peroxisomal membranes and recruit both FIS1 and MFF to the site of membrane elongation. FIS1/MFF then recruits cytosolic DLP1 to its site of action and cooperate with it to mediate the final scission of the peroxisomal membranes [25,29,30,33,58].

2.3. Remerges of the ER in peroxisome biogenesis

In the last 20 years, the concept that the ER acts as a contributor for peroxisome biogenesis has experienced renewed enthusiasm. A common notion for the "growth and division" model is that new peroxisomes can only be formed from pre-existing peroxisomes. The view that peroxisomes multiply solely by growth and division was challenged by the fact that peroxisomes can form *de novo* in cells lacking pre-existing peroxisomes upon re-introduction of the wild-type peroxin. The three peroxins that have been identified to be essential for peroxisomal membrane biogenesis in mammalian cells are PEX3, PEX16, and PEX19 [15–22]. Peroxisomal membrane ghosts are completely absent in the fibroblasts from Zellweger syndrome patients with deletion or non-functional mutation in any of the three genes [15–22]. Upon re-introduction of the gene encoding the corresponding wild-type peroxin, peroxisomal membrane structures could be detected within a short time frame, followed by the restoration of peroxisomal matrix protein import [15–22]. This observation raises the possibility that peroxisomes could be formed from yet-to-be-identified pre-peroxisomal structures or be derived from other organelles.

A number of studies have pointed to the ER as a potential contributor for the *de novo* formation of mammalian peroxisomes. Electron microscopy and 3D image reconstruction from EM tomography of peroxisomal structures in mouse dendritic cells revealed that peroxisomes in these immune cells are surrounded by lamellar-like structures that are enriched with integral membrane proteins PEX3 and PMP70 [59]. These lamellar extensions appeared to be continuous with rough ER [59]. Similar double-membrane structures that were associated with the ER were also observed in CHO cells deficient in PEX6, a component of the peroxisomal AAA ATPase that acts to remove ubiquitinated peroxisomal proteins [60]. However, unlike the lamellar-like structures, all of the double-membrane structures were found to form looped structures surrounding spherical bodies [60]. Similar structures were also observed in the hepatocytes of PEX5 knockout mice [61].

A number of peroxisomal membrane proteins have also been shown to traffic to peroxisomes through the ER, including in particular PEX16, which is involved in the early stages of peroxisome biogenesis [62–64]. When being exogenously expressed, PEX16 is localized to the ER in both *pex19*- and *pex3*-deficient human fibroblast cells, which do not contain any peroxisomal structures, as well as in COS7 cells [62]. *In vitro* membrane targeting assays showed that PEX16 targeted to the ER co-translationally, suggesting that PEX16 could target directly to the ER assumingly by the SRP pathway [62]. Using time-lapse imaging combined with PEX16 tagged with a photoconvertible fluorescent protein, PEX16 has been shown to be able to traffic from the ER to pre-existing peroxisomes [62–64]. Similarly, PEX3 tagged with a *bona fide* SRP recognized ER targeting sequence not only targeted to pre-existing peroxisomes, but also was able to complement *pex3*-deficient cells [63,65].

The mechanism of pre-peroxisomal vesicle/membrane formation from the ER is unclear. However, the formation is unlikely to be similar to vesicle formation from the ER exit sites which is involved in transporting proteins between the ER and Golgi apparatus, since the transport of PEX16 and other PMPs from the ER to peroxisomes was shown to be independent of COPI or COPII [66,67]. This observation has also been interpreted to suggest that peroxisomes are not derived from the ER, but instead formed from pre-peroxisomal structures derived from other sources [66,68]. One of the strongest evidence for the existence of these pre-peroxisomal structures was demonstrated in *H. polymorpha* where the lack of Pex3p and a key autophagy factor Atg1 resulted in the accumulation of reticular and vesicular structures that contain a selective group of peroxins [69]. It is argued that these pre-peroxisomal structures were previously missed as they are rapidly degraded via autophagy [69]. What remains unclear is whether similar structures exist in the mammalian cells and whether the ER is the source of these pre-peroxisomal structures. Given the number of peroxisomal membrane proteins that were demonstrated to target to the ER

Please cite this article as: R. Hua, P.K. Kim, Multiple paths to peroxisomes: Mechanism of peroxisome maintenance in mammals, Biochim. Biophys. Acta (2015), http://dx.doi.org/10.1016/j.bbamcr.2015.09.026